

OFFICE OF NAVAL RESEARCH
CONTRACT N00014-88-C-0118

TECHNICAL REPORT 90-10

**HEMATOLOGIC CHANGES DURING AND FOLLOWING CARDIOPULMONARY
BYPASS AND THEIR RELATIONSHIP TO NON-SURGICAL BLOOD LOSS.
II. ONCOTIC, OPSONIC, AND CLOTTING PROTEINS**

BY

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31 JULY 1990

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19990225030

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM									
1. REPORT NUMBER NBRL, BUSM 90-10	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER									
4. TITLE (and Subtitle) Hematologic changes during and following cardiopulmonary bypass and their relationship to non-surgical blood loss. II. Oncotic, opsonic, and clotting proteins		5. TYPE OF REPORT & PERIOD COVERED									
		6. PERFORMING ORG. REPORT NUMBER									
7. AUTHOR(s) Shukri F Khuri, J Alan Wolfe, Irma O Szymanski, Trevor C Axford, Gina Ragno, Michael Park, Robert Valeri		8. CONTRACT OR GRANT NUMBER(s) N00014-88-C-0118									
9. PERFORMING ORGANIZATION NAME AND ADDRESS Naval Blood Research Laboratory Boston, University School of Medicine 615 Albany St, Boston, MA 02118		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS									
11. CONTROLLING OFFICE NAME AND ADDRESS Naval Medical Research and Development Command Bethesda, MD 20814		12. REPORT DATE 31 July 1990									
		13. NUMBER OF PAGES 42									
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Bureau of Medicine and Surgery Department of the Navy Washington, DC 20372		15. SECURITY CLASS. (of this report) Unclassified									
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE									
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release and sale. Distribution unlimited.											
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)											
18. SUPPLEMENTARY NOTES No 9: Department of Surgery, Brockton/West Roxbury Department of Veterans Affairs Medical Center, Brigham and Women's Hospital, and Harvard Medical School.											
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) <table border="0"> <tr> <td>Blood</td> <td>Blood Loss</td> <td>Shed Blood</td> </tr> <tr> <td>Platelets</td> <td>Cardiopulmonary Bypass</td> <td>Skin Temperature</td> </tr> <tr> <td>Bleeding time</td> <td>Thromboxane B2</td> <td></td> </tr> </table>			Blood	Blood Loss	Shed Blood	Platelets	Cardiopulmonary Bypass	Skin Temperature	Bleeding time	Thromboxane B2	
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Postoperative blood loss was measured after administering the first dose of protamine to reverse the heparin. Fifteen patients had surgical bleeding and were excluded from the data analysis. Preoperatively, abnormally high levels of fibrinogen and C3 were observed in the 85 patients that were analyzed. After correcting for the effect of hemodilution at 20 minutes after the institution of CPB, there was an **increase** in the plasma concentration of the measured proteins except for IgG, IgM, C3, and FVIII-vWF. During the 72-hour post-CPB period, the oncotic and opsonic proteins remained depressed below preoperative levels, while the clotting proteins fibrinogen, FVIIIc, and FVIII-vWF increased to levels significantly higher than preoperative levels. None of the parameters reported in this study correlated by multivariate regression analysis to the blood loss during the 4-hour post-CPB period. Plasma C3 correlated inversely and univariately with the blood loss and with the duration of CPB. The level of D-dimer at 2 hours post-CPB correlated by univariate and multivariate analysis to the template bleeding time measured at the same time. Conclusions: (1) A "plasma refill" phenomenon occurred during the initial period of CPB. (2) Changes in plasma protein levels (including FVIII-vWF) did not predict postoperative blood loss. (3) The relationship between D-dimer and the bleeding time suggested that fibrinolysis might contribute to the platelet dysfunction induced by cardiopulmonary bypass.

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**HEMATOLOGIC CHANGES DURING AND FOLLOWING CARDIOPULMONARY
BYPASS AND THEIR RELATIONSHIP TO NON-SURGICAL BLOOD LOSS**

II. Oncotic, Opsonic, and Clotting Proteins

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Supported by the U.S. Navy (Office of Naval Research Contract
N00014-79-C-0168, with the funds provided by the Naval Medical
Research and Development Command) and by the Richard Warren
Surgical Research and Education Fund.

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ULTRAMINI-ABSTRACT

In 85 patients undergoing cardiopulmonary bypass (CPB), changes in oncotic, opsonic, and coagulation proteins (including FVIII.vWF) as well as changes in PT, PTT, and D-dimer failed to predict postoperative blood loss. The relationship between D-dimer and the bleeding time suggested a role for fibrinolysis in the genesis of CPB-induced platelet dysfunction.

ABSTRACT

Plasma proteins, D-dimer, prothrombin time (PT) and partial thromboplastin time (PTT) were measured before, during, and up to 72 hours following cardiopulmonary bypass (CPB) in 100 adult patients undergoing cardiac surgery. The plasma proteins included total protein and albumin (oncotic proteins), Immunoglobulins G and M, C3, fibronectin (opsonic proteins), and fibrinogen, Factor VIIIC, Factor VIII-vWF protein, antithrombin III, and plasminogen. Postoperative blood loss was measured after administering the first dose of protamine to reverse the heparin. Fifteen patients had surgical bleeding and were excluded from the data analysis. Preoperatively, abnormally high levels of fibrinogen and C3 were observed in the 85 patients that were analyzed. After correcting for the effect of hemodilution at 20 minutes after the institution of CPB, there was an **increase** in the plasma concentration of the measured proteins except for IgG, IgM, C3, and FVIII-vWF. During the 72-hour post-CPB period, the oncotic and opsonic proteins remained depressed below preoperative levels, while the clotting proteins fibrinogen, FVIIIC, and FVIII-vWF increased to levels significantly higher than preoperative levels. None of the parameters reported in this study correlated by multivariate regression analysis to the blood loss during the 4-hour post-CPB period. Plasma C3 correlated inversely and univariately with the blood loss and with the duration of CPB. The level of D-dimer at 2 hours post-CPB correlated by univariate and multivariate analysis to the

template bleeding time measured at the same time. Conclusions:

(1) A "plasma refill" phenomenon occurred during the initial period of CPB. (2) Changes in plasma protein levels (including FVIII-vWF) did not predict postoperative blood loss. (3) The relationship between D-dimer and the bleeding time suggested that fibrinolysis might contribute to the platelet dysfunction induced by cardiopulmonary bypass.

INTRODUCTION

Excessive postoperative bleeding is one of the most frequent complications encountered in cardiac surgery. While at times the source of this bleeding can be an identifiable surgical site, by far the majority of bleeding complications result from a poorly understood abnormality of the hemostatic mechanism precipitated by the extracorporeal circuit (1). This study was undertaken in 100 patients to investigate the hematologic changes which occur during and following cardiopulmonary bypass and to elucidate any direct relationships between these parameters and the extent of non-surgical blood loss following cardiac surgery. The first communication from this study addressed platelet function and described a direct relationship between the bleeding time, temperature, and the magnitude of postoperative non-surgical blood loss (2). This communication addresses the changes in oncotic, opsonic, and clotting cascade proteins as well as D-dimer, the prothrombin time and the partial thromboplastin time and their relationships to non-surgical blood loss.

MATERIALS AND METHODS

One hundred consenting patients (98 males and 2 females) undergoing open-heart surgery at the West Roxbury Department of Veterans Affairs Medical Center were entered into this study. Patient age ranged from 32 to 77 years, averaging 58.7 years.

None of the patients had received antiplatelet or transfusion therapy in the 10-day preoperative period.

Pre-anesthetic medications included intramuscular Innovar (2 ml) and Atropine (0.4-0.6 mg). Anesthesia was induced with Fentanyl and maintained with a combination of Fentanyl, muscle relaxants, and either Halothane or Isoflurane. Patients were placed on extracorporeal circulation using standard techniques and cooled systemically to a temperature range of 30-22 C depending on the complexity of the surgical procedure. The pump was primed with lactated Ringer's solution. During the period of aortic clamping, the heart was selectively cooled with a cardioplegic solution and with topical ice slush to a temperature range of 8-15 C. In all the patients the extracorporeal circuit was identical except for the type of oxygenator which was prospectively randomized between the Bentley Bos 10S bubble oxygenator (American Bentley, Irvine, CA) and the Terumo Capiox II hollow fiber membrane oxygenator (Terumo Corporation, Piscataway, NJ). Heparin was administered prior to institution of cardiopulmonary bypass in an initial dose of 3 mg/kg body weight; subsequent dosages were administered according to the level of the Activated Clotting Time (ACT), which was maintained above 400 seconds throughout the extracorporeal circulation period. At the end of this period heparin was neutralized with protamine sulfate given in a ratio of 0.5 mg protamine to 1.0 mg heparin for the initial heparin dose and 1.0 mg protamine to 1.0 mg heparin for all subsequent heparin doses. Heparin neutralization was also monitored by the ACT. The

systemic temperature was measured intraoperatively by an esophageal thermometer and postoperatively by a rectal thermometer.

Measurement of postoperative blood loss was started intraoperatively when the ACT normalized after the administration of the initial protamine dose, and was achieved by collecting all the blood aspirated from the surgical field and by weighing all the surgical sponges. Postoperatively, an accurate record was kept of the mediastinal drainage until the mediastinal tube was removed, usually on the first or second postoperative day. Fifteen patients in whom excessive bleeding occurred from a specific surgical site were identified and excluded from the data analysis. This report is thus based on data collected from 85 patients with no demonstrable evidence of surgical bleeding. The operations, performed by two surgeons using identical techniques, included the following: 65 isolated coronary artery bypass grafting procedures (CABG), 10 single valve replacements, 9 single valve replacements with CABG, and 1 double valve replacement.

Bleeding times were determined before the induction of anesthesia and then again at 2, 24, 48, and 72 hours following the cardiopulmonary bypass procedure as described and reported elsewhere (2). Samples of arterial blood were obtained before induction of anesthesia, 20 minutes after institution of cardiopulmonary bypass, 2, and 24 hours after cardiopulmonary bypass, and venous blood samples were collected 48 and 72 hours after bypass. These samples were analyzed to determine a total

of 30 hematologic parameters. This communication reports on the levels of 11 different plasma proteins as well as the prothrombin time (PT) and the partial thromboplastin time (PTT). The plasma proteins assayed were the following: (1) Oncotic proteins - total protein (TP), and albumin (Alb); (2) Factor VIII clotting protein (FVIIIc), Factor VIII related-antigen/von Willebrand factor (FVIII-vWF), fibrinogen (Fbg), plasminogen (Plmgn), and antithrombin III (ATIII); (3) Opsonic proteins - Immunoglobulins G and M (IgG, IgM), C3 antigen (C3), and fibronectin (Fbn). D-dimer, a specific fibrin degradation product, was assayed preoperatively, at 2 hours, and at 24 hours post-CPB in a subset of 35 patients.

Detailed records were kept of all the blood products transfused. Patients who did not receive transfusions of fresh frozen plasma or platelets throughout their hospital course were designated as Group A (n=40), and those who did as Group B (n=45). To ascertain the effect of hemodilution secondary to CPB with only crystalloid prime, another group of patients (Group C, n=45), who received no colloid or blood products prior to the 20 minute on-bypass sample point, were examined separately. In this group, the baseline protein value (BL) was compared to the 20 minute on-bypass protein value (BP) and to the on-bypass protein value corrected for dilution (BPC) based on hematocrit (Hct) changes according to the following formula:

$$BPC = BP [(BL \text{ Hct} / BP \text{ Hct} - BL \text{ Hct}) / (1 - BL \text{ Hct})]$$

Laboratory Procedures

Blood samples were collected in K₃EDTA anticoagulant for measurements of hemoglobin concentration using a cyanomethemoglobin technique and a Coulter hemoglobinometer (Coulter Electronics, Edison, NJ). The hematocrit value (Vol %) was measured using the microhematocrit method. Platelet counts were performed by phase microscopy. Total protein and albumin levels were measured by the Biuret reactions method of Kingsley et al (3). The FVIII clotting protein and fibrinogen levels were measured by a clotting assay (4). The FVIII-vWF level was measured by enzyme-immunoassay (5). Plasminogen activity was measured using the chromogenic substrate S-2251 as described by Friberger (6). Antithrombin III was measured by a heparin cofactor assay with chromogenic substrate (7). IgG, IgM, and C3 levels were measured by a nephelometric method for immunochemical determinations (8). Fibronectin was measured by an immunoturbidometric assay (9). Heparin was measured using a chromogenic substrate (10). D-dimer, a breakdown product of fibrinogen, was measured by an enzyme-linked immunoassay using a monoclonal antibody (11). Standard template bleeding times were performed in duplicate using the simplate bleeding time module according to the procedure of Babson and Babson (12).

Data Analysis

The term "post-CPB" in this manuscript denotes the period after the administration of the first dose of protamine to reverse the heparin. The observed and hemodilution-corrected

concentrations of plasma proteins were expressed as mean \pm standard error of the mean (SEM). The paired t-test was used to assess differences between baseline protein concentrations and the observed and hemodilution-corrected concentrations on bypass (Group C). Repeated measures analysis of variance (MANOVA) was used to detect significant changes in a variable throughout the study with respect to time. When a significant change in a protein concentration was noted over time by MANOVA, the paired t-test was used to identify significant differences between adjacent time points within a group. The differences in protein measurements between Group A and Group B were analyzed by the unpaired t-test at discrete time points. A probability level (p value) of 0.05 was considered significant for all analyses. To graphically display the relationship of postoperative blood loss to other measured variables the entire patient population was divided into tierciles based on the range of values for the blood loss in the postoperative period. For blood loss during the initial 4-hour post-CPB period the tiercile levels designated were low: 215-790 ml, n=26; medium: 805-1140 ml, n=26; and high: 1235-2515 ml, n=26. The relationships of postoperative blood loss and the template bleeding time to the other measured variables were univariately analyzed using linear regression, and a significant direct relationship between either the blood loss or the bleeding time and the independent variable was determined at the $p \leq 0.05$ level for the model. These relationships were also examined using a multivariate analysis where the variables predicting the postoperative blood loss and the postoperative

bleeding time were determined using a stepwise multiple general linear regression model. A variable was considered independently significant ($p \leq 0.05$ for the variable) if it improved the overall model's ability to explain the observed variability in the blood loss or the bleeding time when added last to the multivariate general linear model (i.e., Type III Sum of Squares). All the statistical analyses were performed with the SAS statistical package (Cary, North Carolina) on an IBM-compatible personal computer.

RESULTS

Protein Concentrations Before and After Cardiopulmonary Bypass

Table 1 shows the mean (\pm SEM) concentrations of the plasma proteins at baseline and from 2 to 72 hours post cardiopulmonary bypass (CPB) in Group A and Group B patients. Group A patients (n=40) underwent uncomplicated coronary artery bypass graft surgery and did not receive transfusions of either platelets or fresh-frozen plasma during their in-hospital course. Their data are also shown in Figures 1-3. Group B patients (n=45) underwent more complex procedures and received either intraoperative or postoperative transfusions of fresh frozen plasma.

The baseline values of both total protein and albumin were within normal limits. By 2 hours following CPB, total protein and albumin levels decreased significantly ($p < .05$) in both groups and remained depressed compared to baseline up to 72 hours postoperatively.

The baseline values for the opsonic proteins were within normal limits with the exception of C3, which at 148 ± 6 mg/dl for Group A and 135 ± 5 mg/dl for Group B were elevated from a value of 113 mg/dl in normal volunteers assayed in the same laboratory. Following CPB, IgG, IgM, and fibronectin behaved similarly, decreasing progressively ($p < .01$) until 24 hours postoperatively at which point they remained stable for the duration of the study. Fibronectin between 2 and 48 hours postoperatively was significantly lower in Group B than in Group A (Table 1). After its initial decline following CPB, C3 increased progressively ($p < .01$) until 72 hours postoperatively, but it did not return to its baseline value during this period (Fig. 2). C3 was significantly ($p < .05$) less in Group B compared to Group A at 2 hours post-CPB, and remained so for the first 48 hours postoperatively (Table 1).

Among the clotting cascade proteins, the baseline values were all within normal limits except fibrinogen which at 357 ± 15 mg/dl in Group A and 389 ± 19 mg/dl in Group B was elevated from our normal range of 190-300 mg/dl. By 2 hours following CPB, neither FVIIIc nor FVIII-vWF were significantly different from baseline; however, fibrinogen, plasminogen, and antithrombin III decreased significantly ($p < .01$) (Fig. 3). Over the remainder of the study, FVIIIc, FVIII-vWF, and fibrinogen increased progressively, and by 72 hours postoperatively they all were significantly ($p < .01$) higher than baseline. Plasminogen and antithrombin III remained depressed from their baseline levels for the duration of the study (Fig. 3). Random patients ($n=29$)

were assayed for heparin at 2 hours post-CPB. Their plasma contained a negligible level of heparin (0.04 ± 0.01 units/ml).

Protein Concentrations During Cardiopulmonary Bypass

Forty five patients did not receive any blood products throughout the initial hour of cardiopulmonary bypass. They were designated as Group C and were used to study the effects of hemodilution on the concentrations of plasma proteins during cardiopulmonary bypass. The protein concentrations before and at 20 minutes after institution of CPB in Group C are shown in Table 2 and Figure 4. Factor VIIIc and fibrinogen could not be measured on bypass because the patients were fully heparinized. The hemodilution associated with CPB resulted in a decrease in the observed concentrations of all plasma proteins by 20 min on bypass. Table 2 compares the preoperative mean protein values (\pm SEM) in this patient group to both the observed as well as the dilution-corrected protein concentration. The average observed concentration was approximately 50% ($p < .001$) that of the baseline value by 20 minutes on-bypass. After correcting for hemodilution, however, the concentrations of the measured proteins except for C3, IgM, IgG, and FVIII-vWF were significantly increased ($p < 0.01$) compared to baseline. There was a significant decrease in the dilution-corrected concentrations of both C3 ($p < .05$) and IgM ($p < .01$) compared to baseline. IgG and FVIII-vWF both were increased but not to a sufficient degree to obtain statistical significance.

Comparison of Patient Groups A and B

Table 3 provides a comparison between the patients in Groups A and B. The patients were separated into these two groups on the basis of whether or not they had received transfusions of platelets and fresh frozen plasma during their in-hospital course. Group B patients, who received such transfusions, were clearly a more complex group undergoing a longer period of cardiopulmonary bypass and requiring more transfusions of packed red blood cells as well. Group B patients had a significantly larger amount of blood loss during the first 4 hours post-CPB. Despite the increased administration of blood products, including fresh frozen plasma and platelets, the platelet count was significantly lower in Group B and the bleeding time during the first 24 hours postoperatively was significantly increased in this group as compared to Group A (Table 3). The hematocrit was higher in the group receiving less blood product transfusions. As shown in Table 1, the differences noted in protein concentrations between the two groups were a higher level of plasminogen in Group A at 48 hours postoperatively, a lower C3 level at 2, 24, and 48 hours postoperatively in Group B, and a lower level of fibronectin between 2, 24, and 48 hours postoperatively in Group B.

Relationship of the Measured Proteins to Non-surgical Blood Loss Following Cardiopulmonary Bypass

For the patient population in this study most of the postoperative bleeding occurred in the initial 4 hours post-CPB.

Since this period was most reflected by the measurements which were made at the 2-hour post-CPB time point, the total blood loss during the initial 4-hour post-CPB period was chosen to represent the blood loss variable in the regression analysis. Of the proteins, prothrombin time, and partial thromboplastin time measured at 2 hours post-CPB, only plasma C3 related univariately ($p=0.04$) to the 4-hour post-CPB blood loss. As shown graphically in figure 5, the subgroup of patients with lower levels of C3 at 2 hours post-CPB had increased blood loss during the 4-hour post-CPB period. The blood loss during this period did not relate to either the preoperative or the 2-hour post-CPB plasma level of the von Willebrand antigen in this regression analysis. In addition, when the subset of patients whose Factor VIII-vWF level did not increase following bypass was compared to the subset of patients whose Factor VIII-vWF did increase post-CPB, there were no differences in blood loss either in the operating room or postoperatively, as well as no differences in the bleeding time preoperatively and 2 hours post-CPB (Table 4). When a multivariate analysis was performed to elucidate the factors independently predicting postoperative blood loss, none of the measured plasma proteins, including the von Willebrand antigen, C3, and the D-dimer level, either preoperatively or at the 2-hour post-CPB period were independently predictive of the observed 4-hour post-CPB blood loss. As reported previously, only the duration of CPB and the hematocrit 2 hours post-CPB were significant independent predictors of the blood loss during the initial 4-hour post-CPB period (2).

Relationship of Plasma C3 to Duration of Cardiopulmonary Bypass

To elucidate any potential relationship between the total duration of cardiopulmonary bypass and the 2-hour postoperative plasma C3 level, univariate linear regression was performed and demonstrated a significant inverse relationship ($p=0.0001$; Pearson coefficient, $r=-0.44$). Those patients with longer durations of cardiopulmonary bypass exhibited lower plasma C3 levels at the 2-hour post-CPB period.

Relationship of the Measured Proteins to the Bleeding Time Following Cardiopulmonary Bypass

Of the plasma proteins studied, only the D-dimer level at the 2-hour post-CPB time point, measured in 35 of the 85 patients, related univariately ($p=0.01$) to the 2-hour post-CPB bleeding time (figure 6). When the plasma proteins measured at the 2-hour post-CPB period were entered into a multivariate general linear model to determine the independent predictors of the 2-hour post-CPB bleeding time, again, only the plasma D-dimer level was independently predictive of the postoperative bleeding time ($p=0.03$). Those patients with a higher plasma D-dimer level during this period had a greater extension of their bleeding time.

DISCUSSION

This study was designed to elucidate, in part, the hematologic changes which occur in the adult male during and following cardiopulmonary bypass and to explore the role of various hematologic parameters as possible predictors of the extent of non-surgical blood loss during the early period following cardiopulmonary bypass. A specific attempt was made to measure the blood loss and to identify and exclude from the data analysis any patient whose postoperative bleeding could be attributed in part to a surgical event. This communication is the second of two reports describing the results of this study. The first report described the changes in platelet parameters. Significant univariate relationships were elucidated between the blood loss during the initial 4-hour period post-CPB and the duration of cardiopulmonary bypass and the bleeding time at 2 hours post-CPB. In addition, direct relationships were elucidated at 2 hours post-CPB between the bleeding time and the peripheral skin temperature on the one hand, and postoperative blood loss and the sternotomy wound temperature on the other. Multivariate analysis identified the duration of CPB as the most important independent predictor of postoperative blood loss probably because of its influence on platelet parameters and wound temperature (2). In this second report, addressing primarily plasma proteins, the products of fibrinolysis, and the prothrombin and partial thromboplastin clotting assays, only the

plasma C3 level at 2 hours post-CPB related univariately to the initial 4-hour post-CPB blood loss. When these variables were subjected to multivariate analysis, none was independently predictive of blood loss during the initial 4-hour post-CPB period. In addition, when corrected for hemodilution, a net increase in the concentrations of most of the plasma proteins was observed 20 minutes into cardiopulmonary bypass, suggesting a net protein influx from the extravascular space into the intravascular space.

Preoperative protein concentrations: The preoperative plasma concentrations of the oncotic, opsonic, and clotting cascade proteins measured in this study were all within normal limits except for fibrinogen and C3. Fibrinogen was elevated to 377 ± 13 mg/dl from a normal range for our assay of 190-300 mg/dl. These data support the findings of Davey and Parker (13) who reported that 36% of 36 patients studied had elevated fibrinogen levels prior to cardiopulmonary bypass. Other investigators, however, have reported normal levels of plasma fibrinogen levels prior to cardiopulmonary bypass (14, 15, 16). Plasma fibrinogen has been known to be elevated in patients with heart disease (17, 18), and recent data suggest that its measurement in patients might even be predictive of stroke and myocardial infarction (19). Hence, it is quite likely that the elevated baseline level of plasma fibrinogen observed in our patients reflected their underlying heart disease and increased blood viscosity. The elevated baseline level of plasma C3 in our patients cannot be explained. The studies of both Ghandi et al (20), and Boralessa

et al (21) reported normal preoperative C3 values in patients undergoing cardiopulmonary bypass.

Protein changes during cardiopulmonary bypass: The observed on-bypass protein concentration data obtained in this study confirm the observations of other investigators who have reported an approximately 50% reduction in measured plasma protein concentrations secondary to hemodilution (14,20-25). Not all investigators, however, have adequately corrected for hemodilution when comparing pre-bypass and post-bypass protein concentrations. Furthermore, simple comparisons of changes in either hematocrit or albumin concentration can be misleading in a setting where some patients may have received packed red cells and/or plasma transfusions while others may not. In the present study alterations in plasma protein concentrations secondary to crystalloid hemodilution were addressed in a selected subset of patients who received no blood product transfusions prior to the 20 minute on-bypass sample point. Furthermore, the formula utilized in this paper to correct for hemodilution took into account the ratio of the plasma volume to the red blood cell volume within a blood sample. The data, when corrected for hemodilution in this manner, indicated that within 20 minutes following the onset of CPB there was a net **increase** in the intravascular concentration of the plasma proteins that were measured with the exception of IgM, IgG, C3, and FVIII-vWF, the latter proteins being either large molecules or only present in the intravascular space. This immediate "plasma-refill" phenomenon probably represented a net influx of interstitial

proteins into the intravascular space as a consequence of altered capillary permeability and changes in colloid osmotic gradients. Extracorporeal circulation has been shown to cause alterations in vascular integrity which may result in a diffuse perivascular leak in the perioperative period (26). Although a net plasma-refill following cardiopulmonary bypass has not been previously described, a net increase in intravascular albumin concentration following hemorrhage and crystalloid hemodilution has been reported by several investigators (27-31), and several theories and mathematical models have been proposed to explain it (32-36).

Protein changes during the 72-hour period following cardiopulmonary bypass: At 2 hours following cardiopulmonary bypass, both the oncotic and opsonic protein concentrations were decreased by approximately 25% compared to preoperative levels. They continued to be significantly lower than preoperative levels up to 72 hours post-operatively (Figures 1 and 2). Because of the wide variability in blood loss and transfusions of blood and blood products administered to this patient population, it was impossible to calculate the effect of hemodilution on the protein concentrations during the postoperative period in a manner similar to the calculations performed in the selected group of patients during the first 20 minutes of cardiopulmonary bypass. The changes in the oncotic and opsonic proteins during the 2-hour to 72-hour post-CPB period were in part reflective of hemodilution and blood loss since they paralleled the changes observed in the hematocrit during this period (2). Various

investigators have attributed the decrease in opsonic proteins following cardiopulmonary bypass to generalized opsonic consumption during prolonged cardiopulmonary bypass (20, 37), fibronectin-mediated removal of macrocellular aggregates by the reticuloendothelial system (38), protein degradation by proteolytic enzymes (23), and cold-induced precipitation of fibronectin with fibrinogen (39). It is clear, however, that in complex clinical studies such as this one, it is impossible to elucidate with certainty the exact cause for the decrease in the oncotic and opsonic proteins observed in the postoperative period (20, 23, 40). In contrast to the findings of Gandhi and associates (20), who reported that both C3 and fibronectin had recovered significantly by 72 hours following cardiopulmonary bypass, our data showed a sustained decline in the levels of IgG, IgM, and fibronectin in the 72-hour period following cardiopulmonary bypass, with only C3 demonstrating a significant increase during this period (Figure 2). The prompt recovery of opsonic protein concentration in the Gandhi study may have been related to their protocol of pretreatment with methylprednisone prior to cardiopulmonary bypass.

In the clotting cascade proteins, we observed a 25% reduction in fibrinogen concentration 2 hours post-CPB which recovered to baseline by 24 hours postoperatively, and then increased significantly over the following 24 hours. These findings were similar to those previously reported (14, 16), but at odds with those of Moriau et al who reported stable fibrinogen levels over this initial 24-hour period (41). Our observation

that FVIIIc was essentially unchanged 2 hours following CPB and then increased progressively thereafter was also in agreement with the majority of previously published studies (14, 15, 16, 41, 42). It suggested that there was a postoperative increase in the production and release of FVIIIc and FVIII-vWF by the endothelium. Some investigators, however, have failed to document a postoperative increase in the concentrations of these factors (22, 43).

Relationships to postoperative blood loss and bleeding time:

Of the plasma proteins reported in this communication, only C3 at the 2-hour post-CPB period related univariately to the blood loss during the initial 4-hour post-CPB period. Patients with greater postoperative blood loss had lower C3 levels at 2 hours post-CPB (Figure 5). A direct relationship was also elucidated between the length of cardiopulmonary bypass and the level of C3 at 2 hours post-CPB. Hence C3, unlike the total duration of cardiopulmonary bypass, was not an independent predictor of postoperative blood loss, but its relationship to the latter is interesting because it suggests a role for complement activation during cardiopulmonary bypass in the genesis of the hemostatic defect precipitated by extracorporeal circulation.

As previously reported in the first communication from this study (2), the total duration of CPB and the hematocrit at 2 hours post-CPB were the two independent predictors of blood loss during the initial 4-hour post-CPB period. In this study, no plasma protein measured in the postoperative period was independently predictive of the observed postoperative blood

loss. However, the plasma d-dimer level at 2 hours post-CPB was shown to be a significant independent predictor of the bleeding time 2 hours post-CPB. In our study population, patients with higher levels of plasma D-dimer exhibited more prolonged bleeding times in the immediate postoperative period. D-dimer data were available on 35 of the 85 patients in the study - a sample size probably too small to show a direct relationship between d-dimer and blood loss as well. Despite this, the relationship observed between d-dimer and the bleeding time suggests that fibrinolysis also plays a role in the hemostatic abnormality leading to increased blood loss after cardiopulmonary bypass. Complex relationships between platelets and fibrinolysis have been recently identified (44) making it possible to hypothesize that fibrinolysis following cardiac surgery may be related to CPB-induced platelet dysfunction. Furthermore, the finding that the d-dimer level related independently to the postoperative bleeding time following CPB lends credence to recent publications which have advocated the use of aprotinin, a serine protease inhibitor, to reduce blood loss following cardiopulmonary bypass (45, 46). One proposed mechanism of action of aprotinin during cardiopulmonary bypass is the preservation of platelet membrane surface glycoprotein receptors by protease inhibition (47).

Of particular interest in this study was the finding that the preoperative level of FVIII-vWF and its level at the 2-hour post-CPB period did not relate to the blood loss during the initial 4-hour post-CPB period. In a double blind randomized study comparing the effect of the administration of desmopressin

versus placebo to patients undergoing complex cardiac operations, Salzman and associates (48) demonstrated a reduction in both intraoperative and postoperative blood loss in the desmopressin-treated group. They also demonstrated a significantly higher postoperative level of FVIII-vWF in the treated patient group compared to the placebo group and postulated that the beneficial effect of desmopressin was due to its ability to increase the level of plasma von Willebrand protein. Their study also suggested that the preoperative plasma level of FVIII-vWF protein correlated with the magnitude of postoperative blood loss and hence was predictive of which patients would benefit from the administration of desmopressin. Our study, which was conducted in a larger group of patients comprising both complex and routine cardiac operations, failed to elucidate any relationship between postoperative blood loss and either the preoperative or the postoperative plasma levels of FVIII-vWF protein even when analyses similar to those reported by Salzman were performed (Table 4). Likewise, Hackmann and associates did not elicit any postoperative differences in von-Willebrand protein levels and in blood loss between desmopressin and placebo-treated patients undergoing uncomplicated coronary artery bypass surgery (49). Although desmopressin was not administered to patients in our study, our results suggest that if desmopressin is salutary in reducing postoperative blood loss (48, 50), its effect is probably independent of the level of von Willebrand protein.

In summary, this study demonstrated that cardiac surgery patients had high preoperative levels of plasma fibrinogen and C3. It documented that during the initial period of cardiopulmonary bypass there was a net influx from the extravascular space to the intravascular space of all proteins assayed except for IgM, IgG, C3, and FVIII-vWF. In the immediate postoperative period following cardiopulmonary bypass there was a significant decrease in all the proteins assayed, which was sustained over a 72-hour period except for fibrinogen, FVIIIc, and FVIII-vWF, all of which increased to levels significantly higher than preoperative levels. Except for the plasma C3 level at 2 hours post-CPB, changes in proteins did not predict the extent of non-surgical postoperative blood loss. Fibrinolysis may have been an important factor influencing postoperative blood loss and could have contributed to the platelet dysfunction induced by cardiopulmonary bypass.

ACKNOWLEDGEMENTS

The authors acknowledge the contribution of Dr. Philip Lavin who provided all the statistical consultation and advice; Drs Kamal Khabbaz and Joseph Dearani who shared in the analysis and interpretation of the data, Ms Samar Assousa and Mr. Andrew Silverman who collected the specimen and patient data, Ms Carolyn Marquardt and Ms Manisha Patel who performed part of the data analysis, Mrs. Nancy Healey and Mr. Mheir Doursounian who assisted in the editing and preparation of the tables and figures; and Mrs. Donna Kantarges who provided typing and editing services.

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LEGENDS TO FIGURES

Figure 1: Mean concentration of the oncotic proteins before (PRE-OP) and 2 to 72 hours after cardiopulmonary bypass (POST-OP) in 40 patients (Group A) who underwent uncomplicated coronary artery bypass graft surgery and did not receive transfusions of either platelets or fresh-frozen plasma during their in-hospital course.

Figure 2: Mean concentration of the opsonic proteins before (PRE-OP) and 2 to 72 hours after cardiopulmonary bypass (POST-OP) in 40 patients (Group A) who underwent uncomplicated coronary artery bypass graft surgery and did not receive transfusions of either platelets or fresh-frozen plasma during their in-hospital course. IgG = Immunoglobulin G. IgM = Immunoglobulin . C = Complement.

Figure 3: Mean concentration of the coagulation proteins before (PRE-OP) and 2 to 72 hours after cardiopulmonary bypass (POST-OP) in 40 patients (Group A) who underwent uncomplicated coronary artery bypass graft surgery and did not receive transfusions of either platelets or fresh-frozen plasma during their in-hospital course. FVIII = Factor VIII. c = clotting. vWF = Von Willebrand Factor. AT = Antithrombin.

Figure 4: Mean protein concentrations before (PRE-OP) and 20 minutes after the institution of cardiopulmonary bypass in 45 patients (Group C) who did not receive any blood products throughout the initial hour of cardiopulmonary bypass. The open barographs show the preop values, the solid barographs show the measured values during cardiopulmonary bypass, and the hatched barographs show the values during cardiopulmonary bypass when corrected for hemodilution (see text for the formula used in this correction). The asterisk indicates a significant ($P < 0.05$) difference from preop. TP = Total protein. ALB = Albumin. FBG = Fibrinogen. Plmgn = Plasminogen. Other abbreviations as in Figures 2 and 3.

Figure 5: Mean \pm standard error of plasma C3 at 2 hours following cardiopulmonary bypass in three patient groups (N= 24 in each) divided according to tierciles of blood loss during the 4-hour period post-cardiopulmonary bypass. The patient group with the least amount of blood loss (low tiercile) had the highest level of C3.

Figure 6: The relationship between the bleeding time and the plasma d-dimer antigen 2 hours post-cardiopulmonary bypass in 35 patients in whom measurements of d-dimer were obtained.

TABLE 1: PLASMA PROTEIN CONCENTRATIONS (MEANS \pm SEM) IN GROUPS A AND B BEFORE AND UP TO 72 HOURS AFTER CARDIOPULMONARY BYPASS

TIME GROUP	ALBUMIN (g/dl)		TOTAL PROTEIN (g/dl)		C3 (mg/dl)		IgG (mg/dl)		IgM (mg/dl)		FBN (ug/ml)	
	A	B	A	B	A	B	A	B	A	B	A	B
PRE-OP	3.50 \pm 0.07	3.34 \pm 0.09	6.42 \pm 0.12	6.23 \pm 0.16	148 \pm 6	135 \pm 5	977 \pm 41	939 \pm 33	136 \pm 13	129 \pm 10	425 \pm 21	393 \pm 20
POST-OP (HRS)
2 HOURS	2.80 \pm 0.09	2.70 \pm 0.08	4.82 \pm 0.14	4.69 \pm 0.11	90 \pm 4	76 \pm 2	681 \pm 37	644 \pm 24	81 \pm 9	75 \pm 6	318 \pm 17	275 \pm 14

24 HOURS	2.98 \pm 0.10	2.85 \pm 0.12	4.98 \pm 0.14	4.62 \pm 0.14	80 \pm 4	65 \pm 3	605 \pm 32	572 \pm 23	67 \pm 7	60 \pm 5	258 \pm 12	215 \pm 12

48 HOURS	2.88 \pm 0.09	2.87 \pm 0.09	5.15 \pm 0.15	4.84 \pm 0.12	94 \pm 4	81 \pm 3	579 \pm 34	578 \pm 23	66 \pm 7	63 \pm 4	273 \pm 17	230 \pm 13

72 HOURS	2.76 \pm 0.10@	2.75 \pm 0.08@	5.24 \pm 0.19@	4.89 \pm 0.12@	107 \pm 5@	99 \pm 4@	599 \pm 32@	599 \pm 21@	71 \pm 7@	64 \pm 4@	287 \pm 19@	252 \pm 16@

TIME GROUP	FVIIIc (%N)		FVIII-vWF (u/ml)		PLMGN (%)		ATIII (%)		FBG (mg/dl)	
	A	B	A	B	A	B	A	B	A	B
PRE-OP	109 \pm 11	123 \pm 7	1.62 \pm 0.13	1.72 \pm 0.11	105 \pm 3	100 \pm 3	105 \pm 2	103 \pm 3	357 \pm 15	389 \pm 19
POST-OP (HRS)
2 HOURS	102 \pm 9	103 \pm 9	1.77 \pm 0.16	1.76 \pm 0.11	78 \pm 2	73 \pm 2	78 \pm 2	73 \pm 3	266 \pm 18	250 \pm 18

24 HOURS	121 \pm 10	109 \pm 8	1.94 \pm 0.19	2.03 \pm 0.17	67 \pm 2	61 \pm 2	74 \pm 3	70 \pm 2	356 \pm 17	340 \pm 17

48 HOURS	158 \pm 12	141 \pm 7	2.65 \pm 0.24	2.43 \pm 0.22	68 \pm 2	61 \pm 2	76 \pm 2	73 \pm 3	511 \pm 24	483 \pm 22

72 HOURS	181 \pm 15@	165 \pm 10@	2.70 \pm 0.22@	2.79 \pm 0.18@	72 \pm 3@	66 \pm 2@	79 \pm 3@	77 \pm 3@	581 \pm 25@	566 \pm 26@

* p<0.05, ** p<0.01, *** p<0.001

- Group A patients received no platelet or fresh frozen plasma transfusions throughout their hospital care.

- Group B patients received both platelet and fresh frozen plasma transfusions.

- * within vertical columns denotes significance compared to preceding value by paired t-test

- * between column A and column B for a variable denotes significance between Groups A and B by unpaired t-test at that time point

- @ at 72 hours denotes a significant difference from preop by paired t-test

- C3, complement; IgG, immunoglobulin G; IgM, immunoglobulin M; FBN, fibrinogen;

FVIIIc, Factor VIII clotting protein; FVIII-vWF, Factor VIII related antigen/vonWillebrand factor;

PLMGN, plasminogen; ATIII, anti-thrombin III; FBG, fibrinogen.

TABLE 2: COMPARISON OF OBSERVED AND DILUTION-CORRECTED PROTEIN CONCENTRATIONS DURING CARDIOPULMONARY BYPASS IN GROUP C (n=48)

PROTEINS	PRE-OP	ON-BP	
		OBSERVED	CORRECTED
ALBUMIN (g/dl)	3.4 ± 0.1	1.8 ± 0.1 **	4.0 ± 0.1 **
TP (g/dl)	6.4 ± 0.1	3.3 ± 0.1 **	7.2 ± 0.2 **
FVIII-vWF (u/ml)	1.6 ± 0.1	0.8 ± 0.1 **	1.7 ± 0.1 NS
ATIII (%)	103 ± 2	60 ± 3 **	132 ± 7 **
PLMGN (%)	101 ± 3	55 ± 2 **	119 ± 4 **
C3 (mg/dl)	143 ± 5	61 ± 2 **	134 ± 5 *
IgG (mg/dl)	989 ± 31	453 ± 18 **	997 ± 39 NS
IgM (mg/dl)	143 ± 12	53 ± 4 **	119 ± 10 **
FBN (ug/ml)	396 ± 18	216 ± 13 **	466 ± 21 **

- * $p < 0.01$, ** $p \leq 0.0001$ Observed/Corrected values compared to Pre-op values
- NS, non-significant
- PRE-OP, prior to anesthesia; ON-BP, during cardiopulmonary bypass.
- TP, total protein; FVIII-vWF, Factor VIII related antigen/vonWillebrand factor; ATIII, anti-thrombin III; PLMGN, plasminogen; C3, complement; IgG, immunoglobulin G; IgM, immunoglobulin M; FBN, fibronectin.

TABLE 3: CHARACTERISTICS OF THE TWO PATIENT GROUPS

	GROUP A (n=40)	GROUP B (n=45)	<i>p</i>
Operation: CABG	36	29	
Valve±CABG	4	16	
Duration of CPB (minutes)	114±6	160±9	0.0001
Blood Loss During the 4-hour Post-CPB Period (ml)	838±62	1230±75	0.0001
Transfusions (units):			
Packed RBCs	3.7±0.3	6.8±0.6	0.0001
Fresh Frozen Plasma	0.0±0.0	3.6±0.5	0.0001
Platelets	0.0±0.0	6.1±1.1	0.0001
Hematocrit (vol %):			
Pre-CPB	37.7±0.5	38.7±0.7	0.22
2 hours Post-CPB	33.9±0.8	31.8±0.6	0.03
24 hours Post-CPB	30.8±0.7	28.9±0.8	0.08
Platelet Count (x10 ³ /mm ³):			
Pre-CPB	214±10	194±8	0.10
2 hours Post-CPB	141±7	113±6	0.003
24 hours Post-CPB	134±7	110±7	0.02
Bleeding Time (minutes):			
Pre-CPB	7.5±0.4	8.2±0.5	0.22
2 hours Post-CPB	12.4±0.7	14.7±0.7	0.02
24 hours Post-CPB	10.3±0.6	12.0±0.6	0.05
Prothrombin Time (seconds):			
Pre-CPB	10.4±0.1	10.6±0.1	0.38
2 hours Post-CPB	12.5±0.3	12.9±0.2	0.29
24 hours Post-CPB	11.8±0.4	11.9±0.3	0.89
Partial Thromboplastin Time (seconds):			
Pre-CPB	31.5±3.7	31.2±0.7	0.77
2 hours Post-CPB	37.3±1.2	39.2±1.1	0.24
24 hours Post-CPB	38.5±1.0	39.1±1.1	0.68

TABLE 4: RELATIONSHIP BETWEEN FVIII-vWF, BLOOD LOSS, AND BLEEDING TIME BY GROUPING PATIENTS ACCORDING TO RELATIVE CHANGE IN FVIII-vWF FROM PRE-OP TO 2 HOURS AFTER BYPASS

		<u>FVIII-vWF</u>		<u>p *</u>
		<u>PRE <1.8 u/ml</u> <u>2 HR<1.8 u/ml</u> (n=25)	<u>PRE <1.8 u/ml</u> <u>2 HR>1.8 u/ml</u> (n=12)	
OR BLOOD LOSS	(ml)	720±75	749±105	0.8
TOTAL BLOOD LOSS	(ml)	1885±159	1833±187	0.8
BT PRE-OP	(min)	7.3±0.4	8.9±1.2	0.2
BT 2 HR	(min)	12.7±0.9	13.6±1.4	0.6

* Student t-test

- FVIII-vWF = Plasma Factor VIII-von Willebrand
- BT Pre-Op = Bleeding Time Preoperatively
- BT 2 HR = Bleeding Time 2 Hours Postoperatively

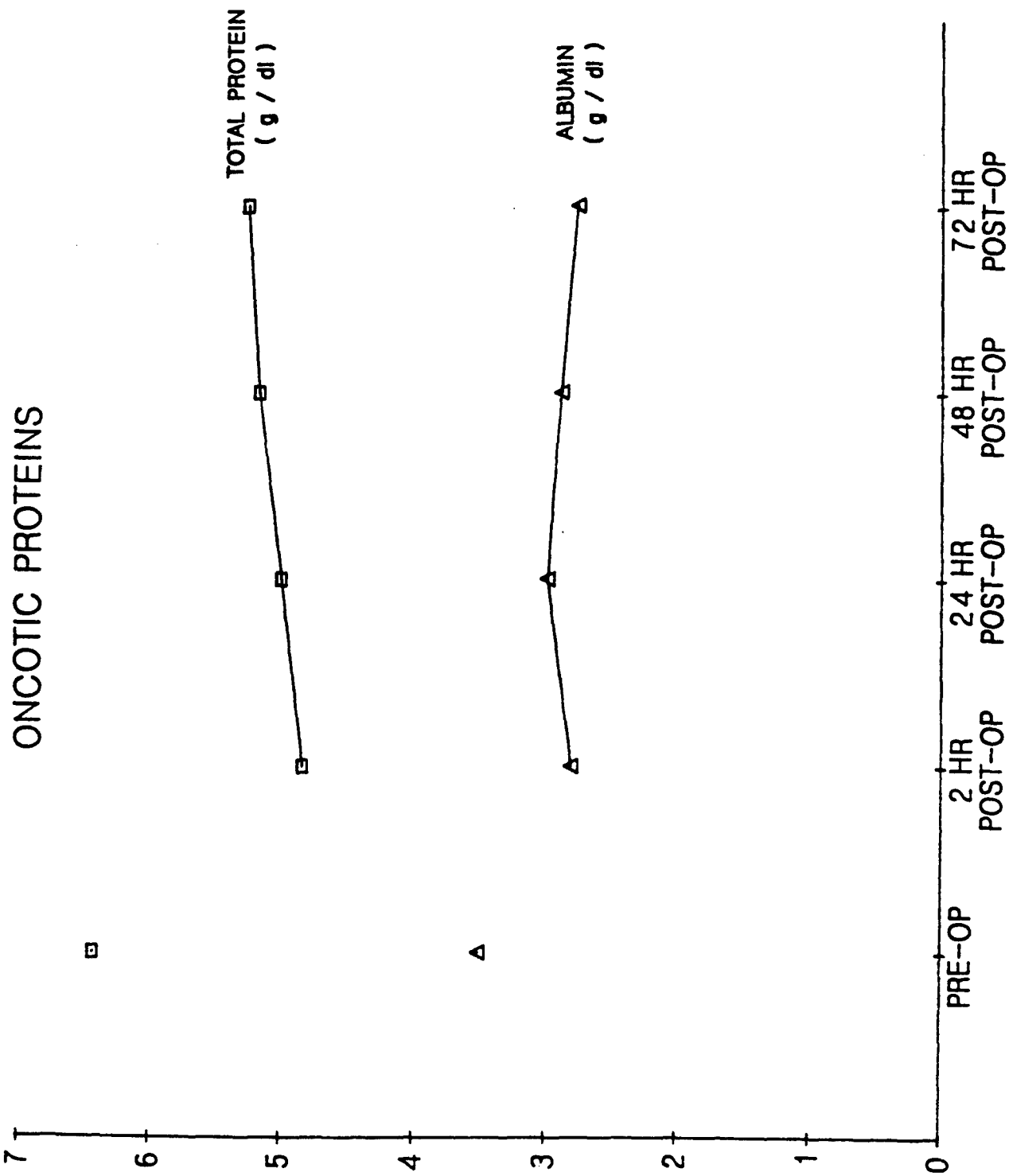


Fig. 1

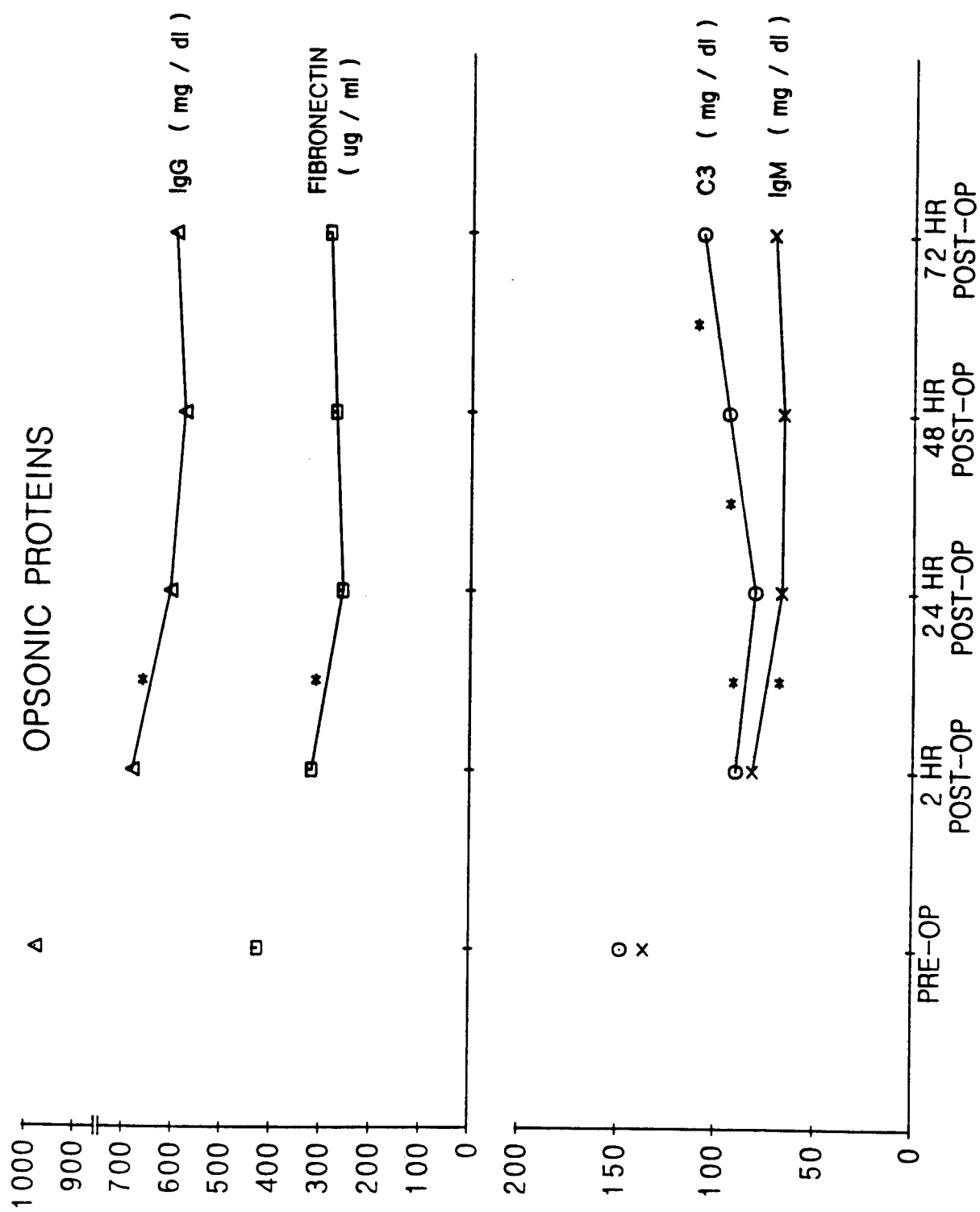


Fig. 2

CLOTTING PROTEINS

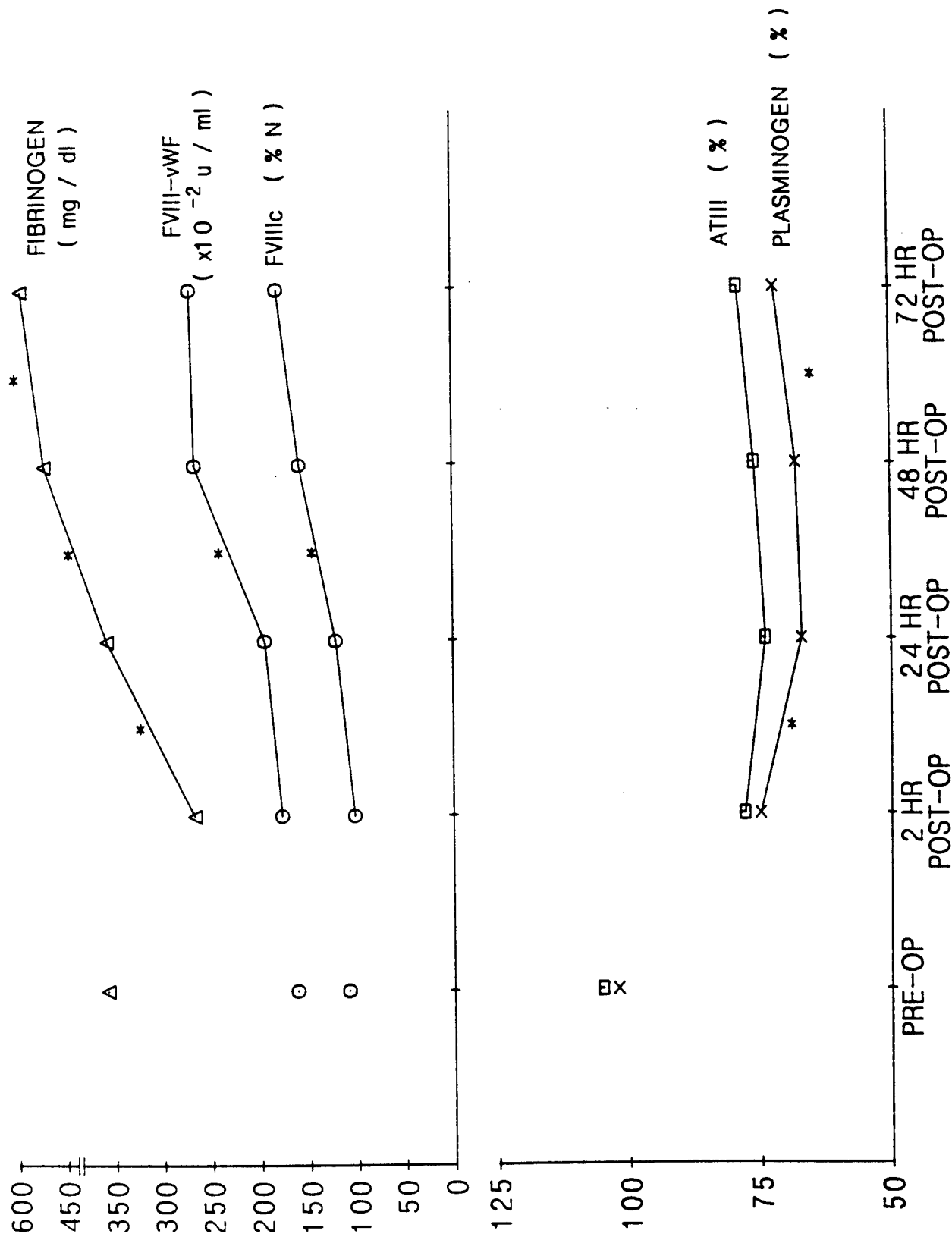


Fig. 3

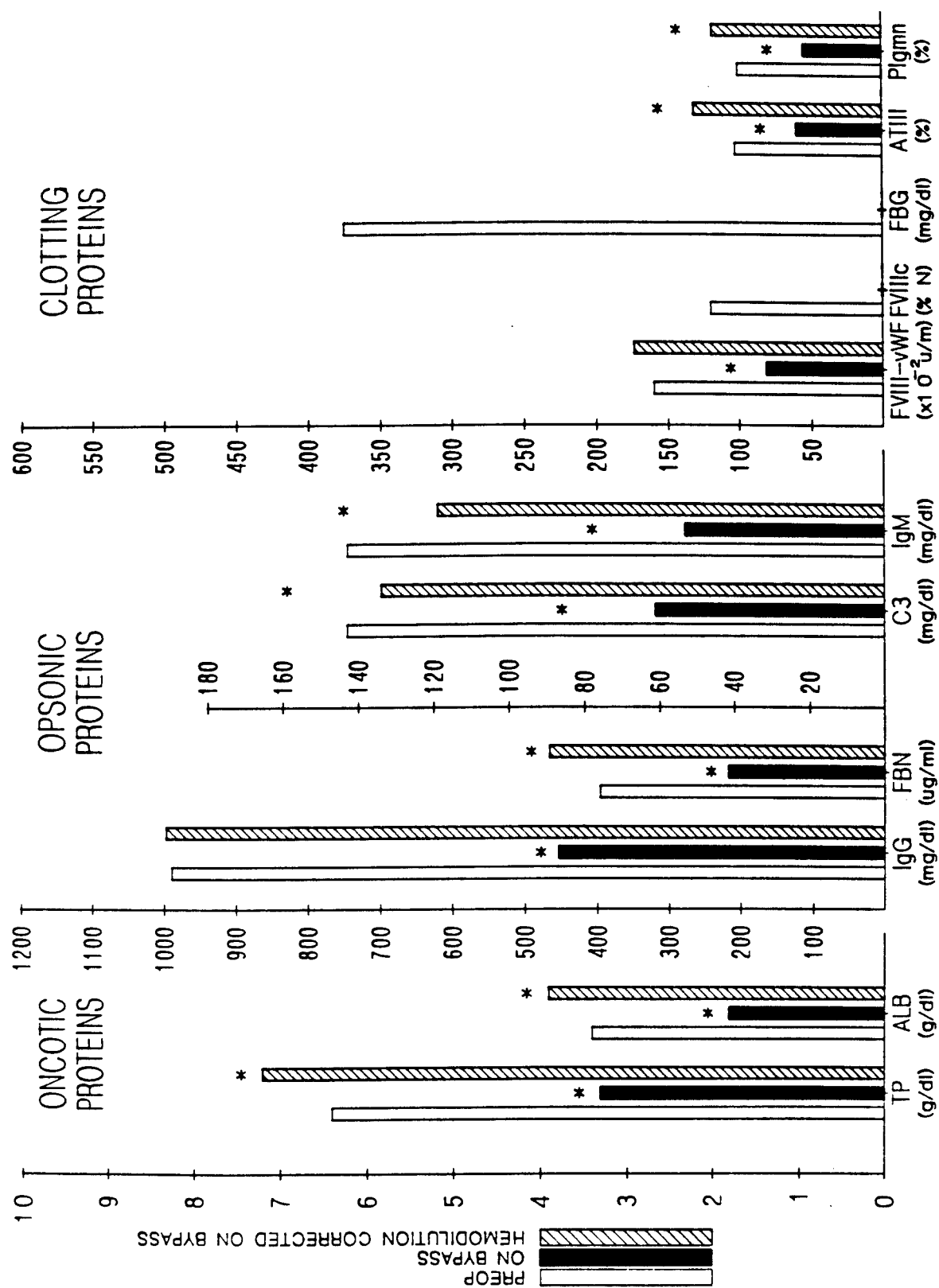


Fig. 4

TIERCILES OF BLOOD LOSS DURING THE 4-HOUR PERIOD POST-CPB

